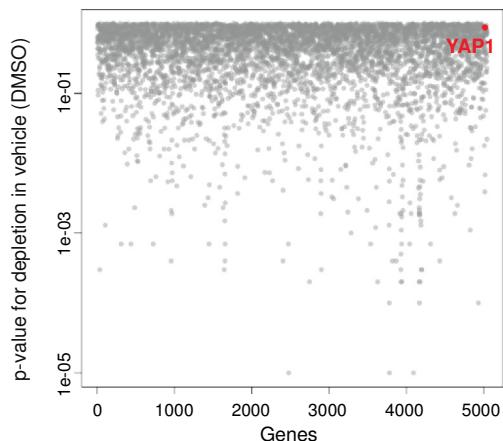
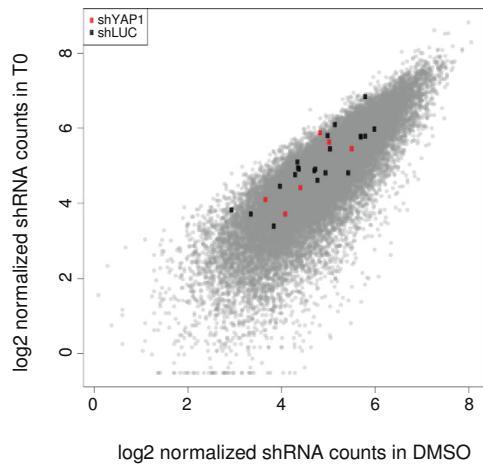


Supplementary Figure 1.

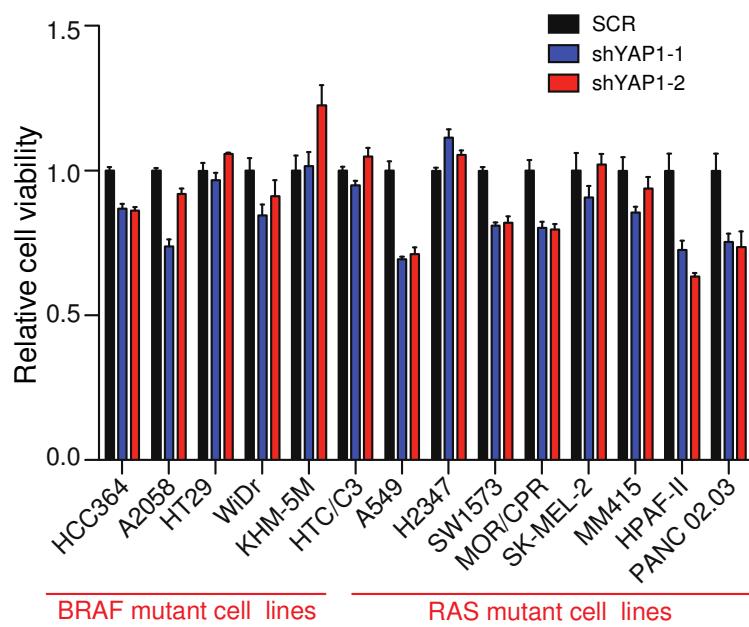
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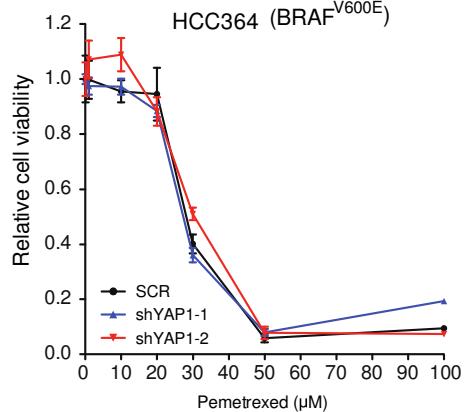
Supplementary Figure 1

Effects of *YAP1* inhibition on cell growth.

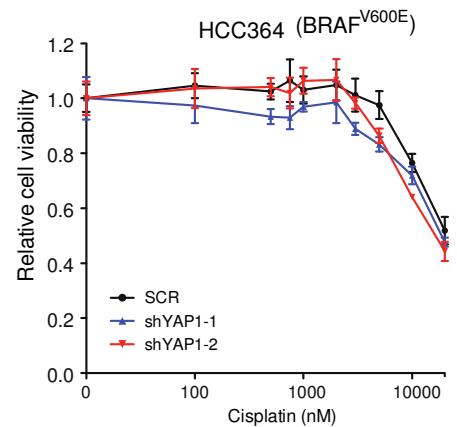
(a) Primary screen data showing that the *YAP1* gene was not depleted over 10 d of vehicle (DMSO) treatment in HCC364 cells (DMSO versus T0). **(b)** Primary screening data showing that shRNAs targeting *YAP1* were not depleted over 10 d of vehicle (DMSO) treatment in HCC364 cells (DMSO versus T0). **(c)** Effect of *YAP1* knockdown using two independent shRNAs on cell growth in HCC364, A2058, HT29, WiDr, KHM-5M, HTC/C3, A549, H2347, SW1573, MOR/CPR, SK-MEL-2, MM415, HPAF-II and PANC02.03 cells, measured at 3 d by CellTiter-Glo assay.

Supplementary Figure 2.

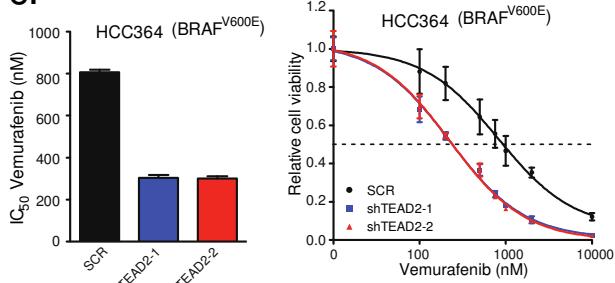
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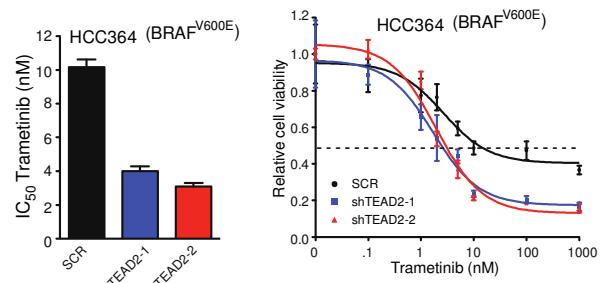
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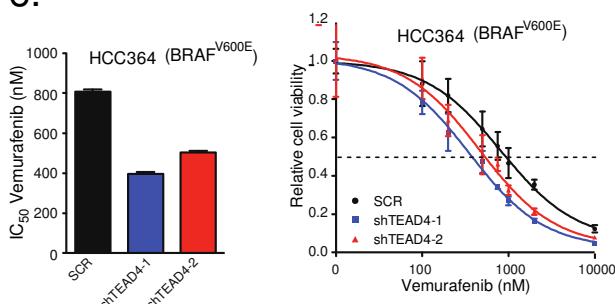
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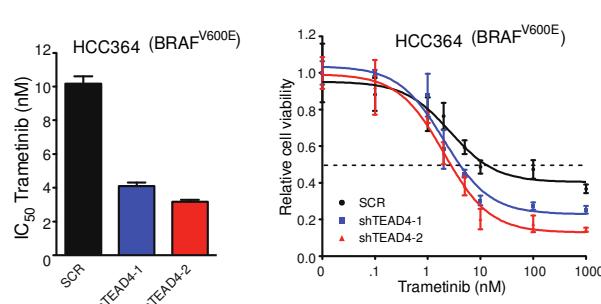
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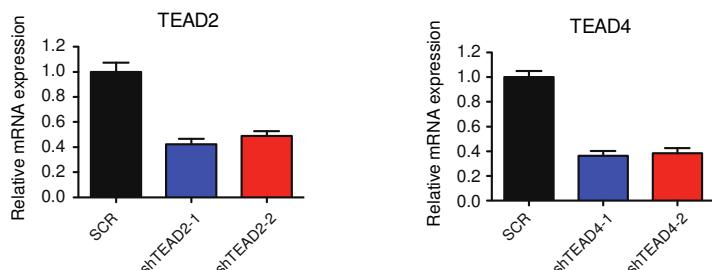
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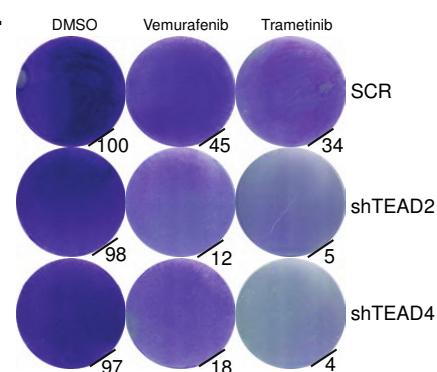
f.



g.



h.



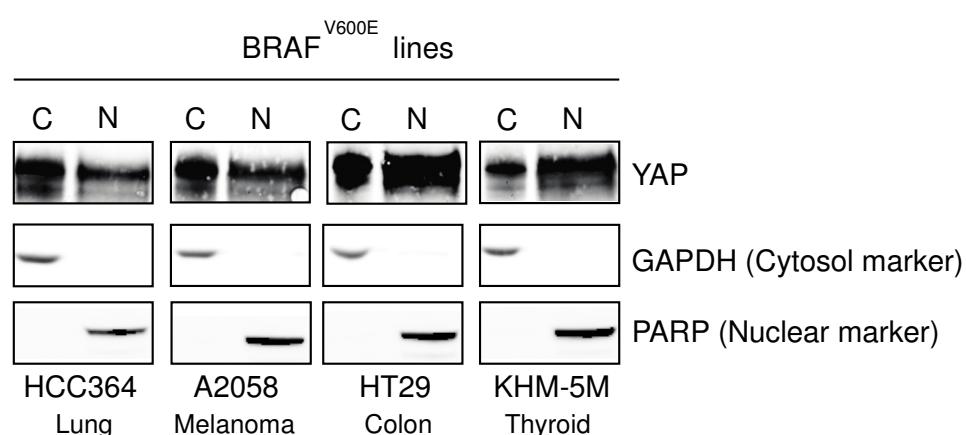
Supplementary Figure 2

The effects of YAP silencing were specific to targeted inhibition of RAF-MEK signaling and are through YAP transcriptional activity.

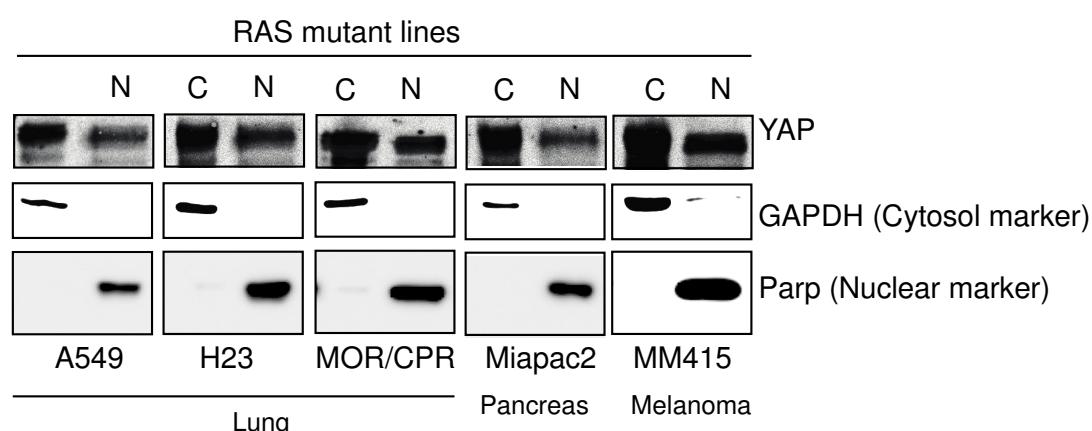
(a) Effects of *YAPI* knockdown using two independent shRNAs on pemetrexed sensitivity in HCC364 lung cancer cells. (b) Effects of *YAPI* knockdown using two independent shRNAs on cisplatin sensitivity in HCC364 lung cancer cells. (c) Effects of *TEAD2* knockdown using two independent shRNAs on vemurafenib sensitivity in HCC364 lung cancer cells (shown are the IC₅₀ and relative cell viability). (d) Validation of the effects of *TEAD2* knockdown on trametinib sensitivity in HCC364 BRAF-mutant lung cancer cells (shown are the IC₅₀ and cell viability). (e) Effects of *TEAD4* knockdown using two independent shRNAs on vemurafenib sensitivity in HCC364 lung cancer cells (shown are the IC₅₀ and relative cell viability). (f) Validation of the effects of *TEAD4* knockdown on trametinib sensitivity in HCC364 BRAF-mutant lung cancer cells (shown are the IC₅₀ and cell viability). (g) mRNA expression of *TEAD2* and *TEAD4* in cells expressing scrambled control shRNA or shRNA to *TEAD2* or *TEAD4*. (h) Effects of *TEAD2* and *TEAD4* knockdown on vemurafenib and trametinib sensitivity in HCC364 BRAF-mutant lung cancer cells (shown is 7-d cell growth assessed by crystal violet staining assays, with quantification of the effects on viability under each condition).

Supplementary Figure 3.

a.



b.

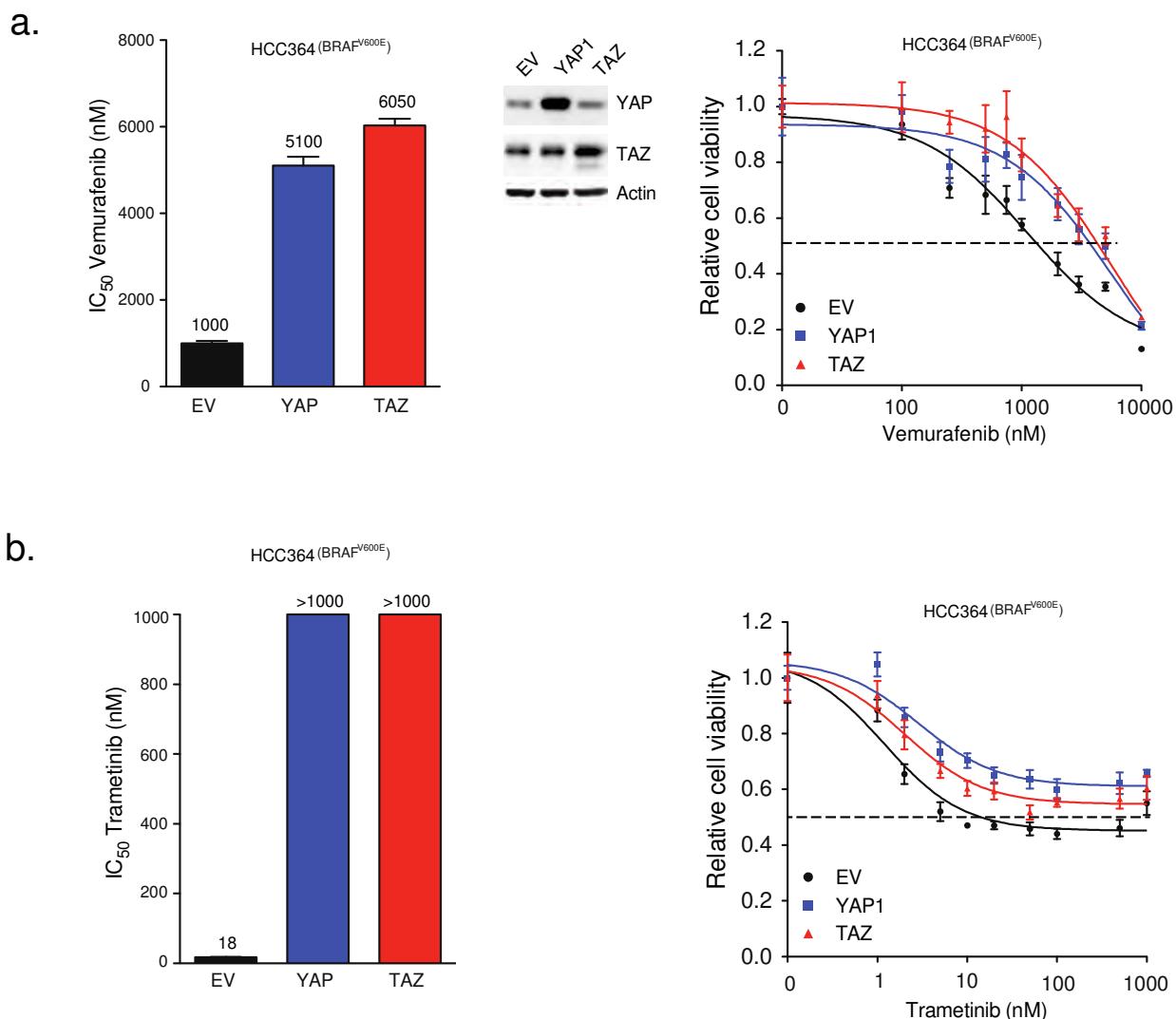


Supplementary Figure 3

Nuclear YAP expression in BRAF- and RAS-mutant cancer cell lines.

Nuclear/cytoplasmic fractionation and immunoblot analysis of the indicated proteins in (a) BRAF V600E–mutant cancer cell lines and (b) RAS-mutant cancer cell lines. Data represent three independent experiments.

Supplementary Figure 4.

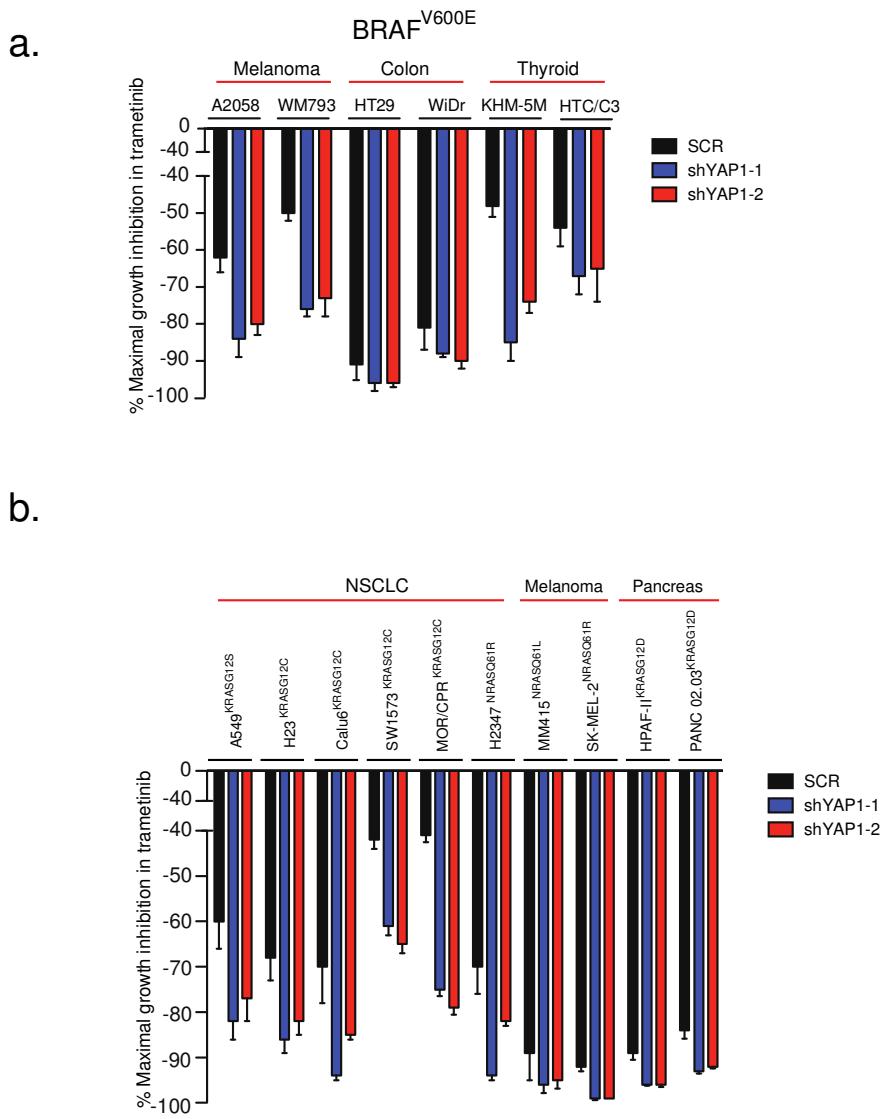


Supplementary Figure 4

Exogenous expression of YAP or TAZ promotes resistance to RAF-MEK inhibition.

- (a) Effects of *YAPI* or *TAZ* overexpression on vemurafenib sensitivity in HCC364 lung cancer cells (shown are the IC₅₀ and relative cell viability).
(b) Effects of *YAPI* or *TAZ* overexpression on trametinib sensitivity in HCC364 lung cancer cells (shown are the IC₅₀ and relative cell viability).

Supplementary Figure 5.

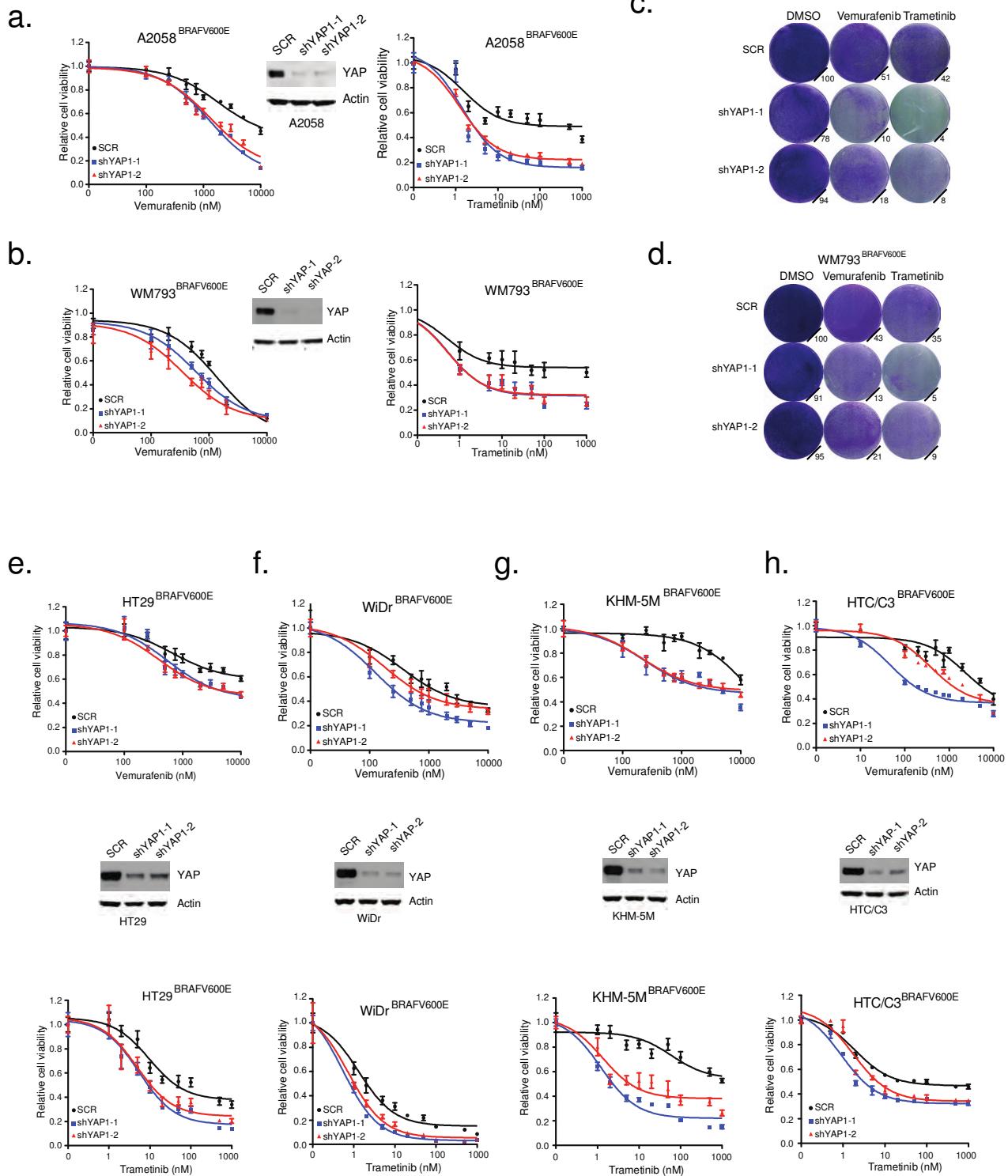


Supplementary Figure 5

Increase in maximal growth inhibition upon trametinib treatment in *YAP1*-depleted cells.

(a) Effects of *YAP1* knockdown on trametinib sensitivity in the indicated BRAF-mutant cell lines, shown as percentage of maximal growth inhibition ($n = 4$, +s.e.m. for all cell viability data shown). (b) Effects of *YAP1* knockdown on trametinib sensitivity in the indicated RAS-mutant cell lines, shown as percentage of maximal growth inhibition ($n = 4$, +s.e.m. for all cell viability data shown).

Supplementary Figure 6.

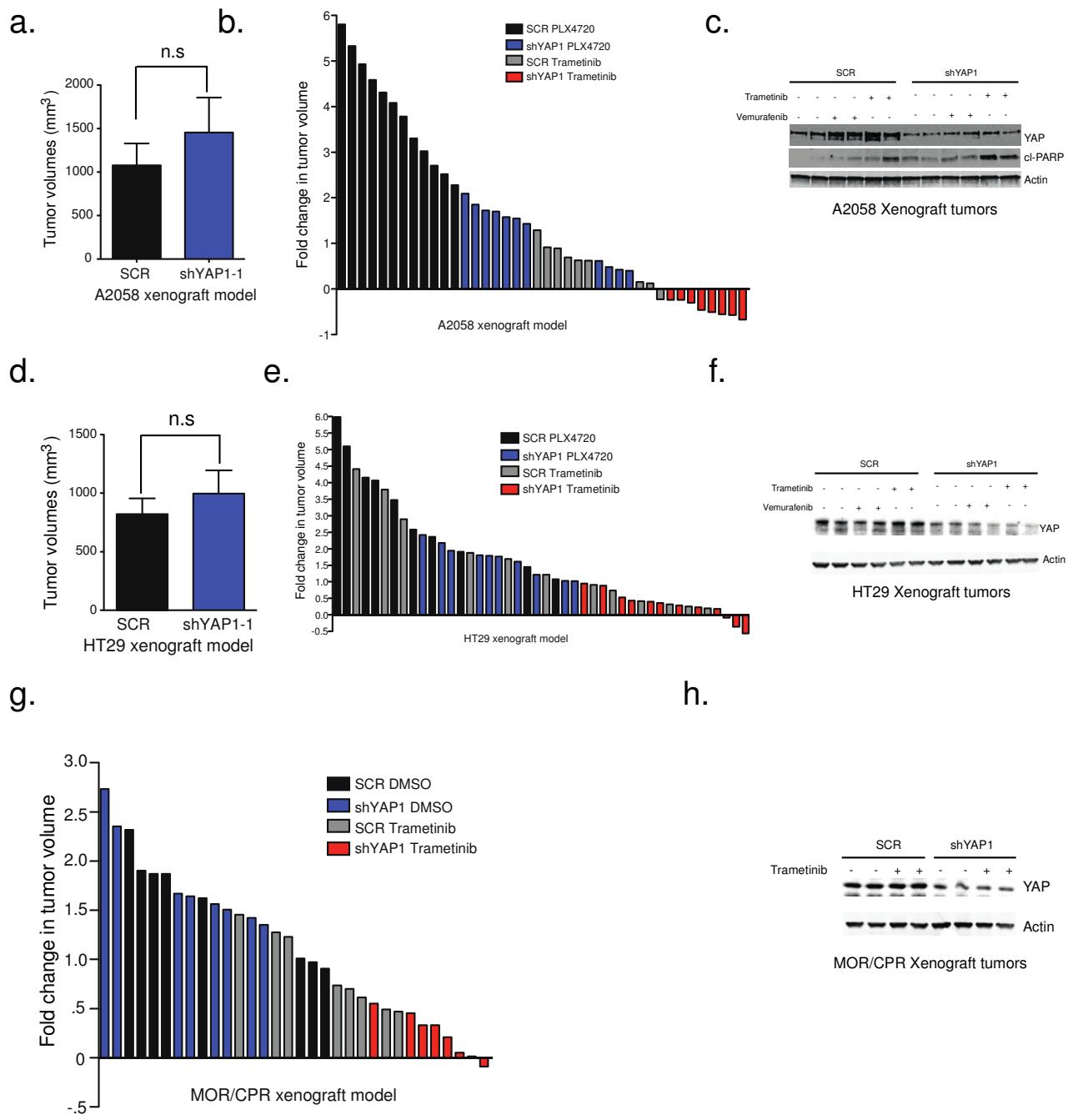


Supplementary Figure 6

***YAP1* knockdown sensitizes cancer cells to RAF-MEK inhibition across multiple tumor types.**

(a,b) Effects of *YAP1* knockdown using two independent shRNAs on vemurafenib and trametinib sensitivity in **(a)** A2058 and **(b)** WM793 BRAF V600E-mutant melanoma cancer cells. **(c,d)** Effects of *YAP1* knockdown on vemurafenib and trametinib sensitivity in **(c)** A2058 and **(d)** WM793 BRAF V600E-mutant melanoma cancer cells (shown is 7-d cell growth assessed by crystal violet staining assays, with quantification of the effects on viability under each condition). **(e,f)** Effects of *YAP1* knockdown using two independent shRNAs on vemurafenib and trametinib sensitivity in **(e)** HT29 and **(f)** WiDr BRAF V600E-mutant colon cancer cells. **(g,h)** Effects of *YAP1* knockdown using two independent shRNAs on vemurafenib and trametinib sensitivity in **(g)** KHM-5M and **(h)** HTC/C3 BRAF V600E-mutant thyroid cancer cells.

Supplementary Figure 7.



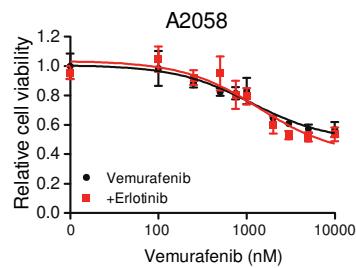
Supplementary Figure 7

***YAP1* inhibition sensitizes tumors to RAF-MEK inhibition *in vivo*.**

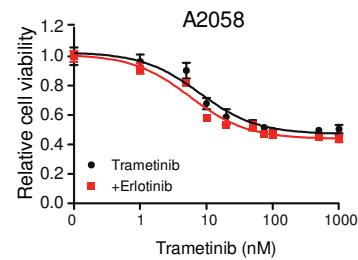
(a) Average tumor volume of xenografts generated from A2058 cells infected with either scrambled control shRNA or *YAP1* shRNA ($n = 10/\text{group}$, $\pm\text{s.e.m.}$). **(b)** Waterfall plot indicating fold change in tumor volume upon vemurafenib or trametinib treatment of A2058 xenografts infected with either scrambled control shRNA or *YAP1* shRNA ($n = 8\text{--}12/\text{group}$, $\pm\text{s.e.m.}$). **(c)** Immunoblot analysis for each indicated protein in lysates from representative A2058 xenograft tumors. **d**, Average tumor volume of xenografts generated from HT29 cells infected with either scrambled control shRNA or *YAP1* shRNA ($n = 10/\text{group}$, $\pm\text{s.e.m.}$). **(e)** Waterfall plot indicating fold change in tumor volume upon vemurafenib or trametinib treatment of HT29 xenograft model infected with either scrambled control shRNA or *YAP1* shRNA ($n = 8\text{--}12/\text{group}$, $\pm\text{s.e.m.}$). **(f)** Immunoblot analysis for each indicated protein in lysates from representative HT29 xenograft tumors. **(g)** Waterfall plot indicating fold change in tumor volume upon trametinib treatment of MOR/CPR xenograft model infected with either scrambled control shRNA or *YAP1* shRNA ($n = 8\text{--}12/\text{group}$, $\pm\text{s.e.m.}$). **(h)** Immunoblot analysis for each indicated protein in lysates from representative MOR/CPR xenograft tumors.

Supplementary Figure 8.

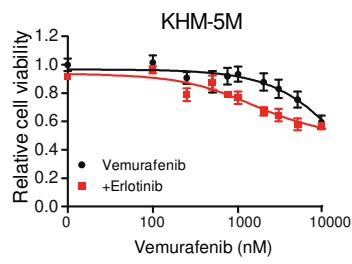
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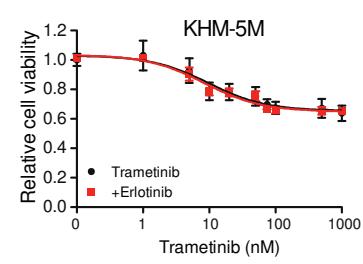
b.



c.



d.



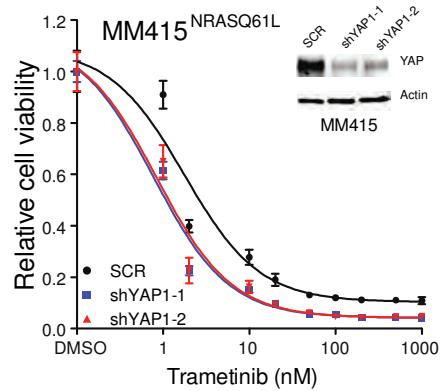
Supplementary Figure 8

Effect of treatment with the EGFR kinase inhibitor erlotinib on RAF-MEK inhibitor sensitivity.

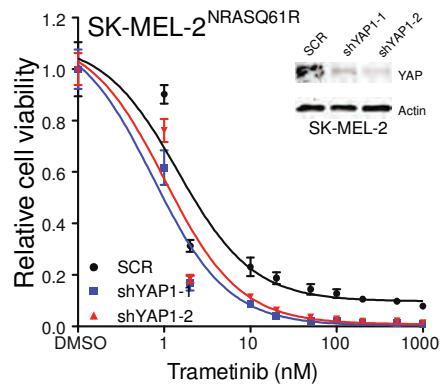
(a,b) Effect of treatment with the EGFR kinase inhibitor erlotinib on **(a)** vemurafenib and **(b)** trametinib sensitivity in A2058 BRAF V600E–mutant melanoma cells. **(c,d)** Effect of EGFR kinase inhibitor erlotinib on **(c)** vemurafenib and **(d)** trametinib sensitivity in KHM-5M BRAF V600E–mutant thyroid cancer cells.

Supplementary Figure 9.

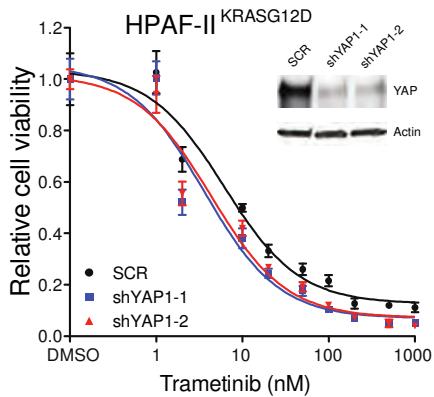
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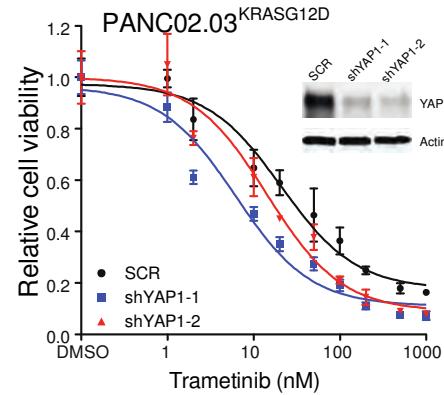
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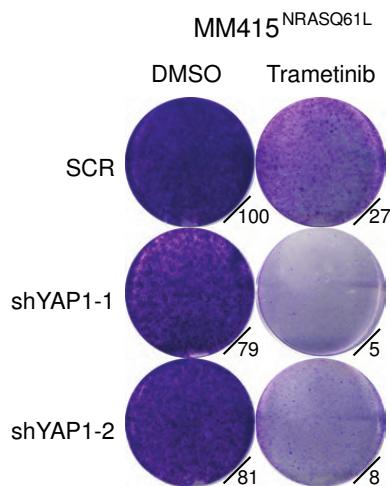
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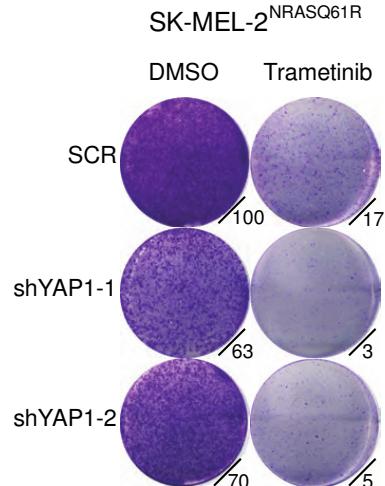
d.



e.



f.

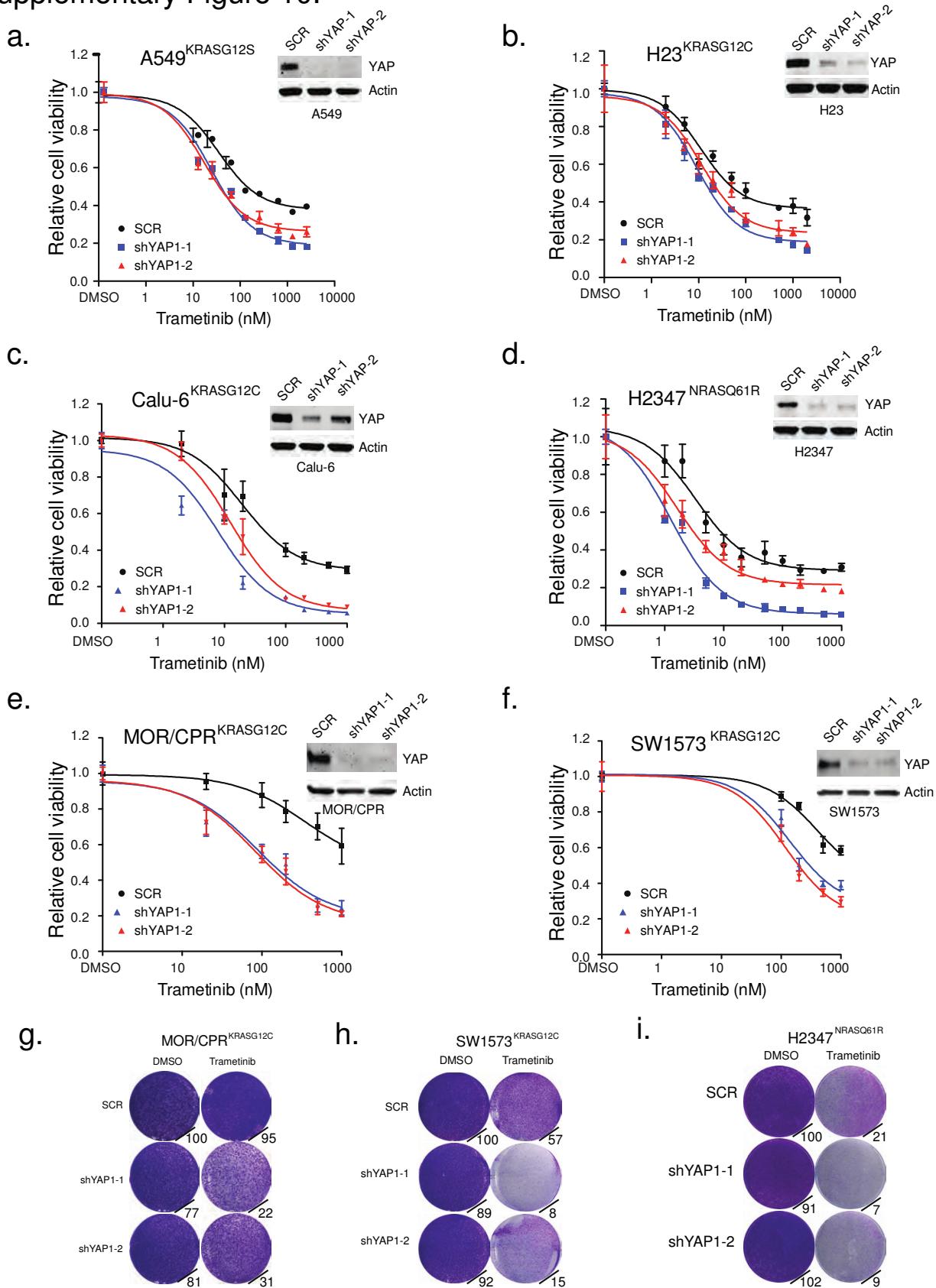


Supplementary Figure 9

***YAP1* knockdown sensitizes RAS-mutant melanoma and pancreatic cancer cells to MEK inhibition.**

(a,b) Effects of *YAP1* knockdown using two independent shRNAs on trametinib sensitivity in **(a)** MM415 NRAS Q61L–mutant, **(b)** SK-MEL-2 NRAS Q61R–mutant melanoma cells. **(c,d)** Effects of *YAP1* knockdown using two independent shRNAs on trametinib sensitivity in **(c)** HPAF-II KRAS G12D–mutant and **(d)** Panc 02.03 KRAS G12D–mutant pancreatic cancer cells. **(e,f)** Effects of *YAP1* knockdown using two independent shRNAs on trametinib sensitivity in **(e)** MM415 NRAS Q61L–mutant and **(f)** SK-MEL-2 NRAS Q61R–mutant melanoma cells (shown is 7-d cell growth assessed by crystal violet staining assays, with quantification of the effects on viability under each condition).

Supplementary Figure 10.

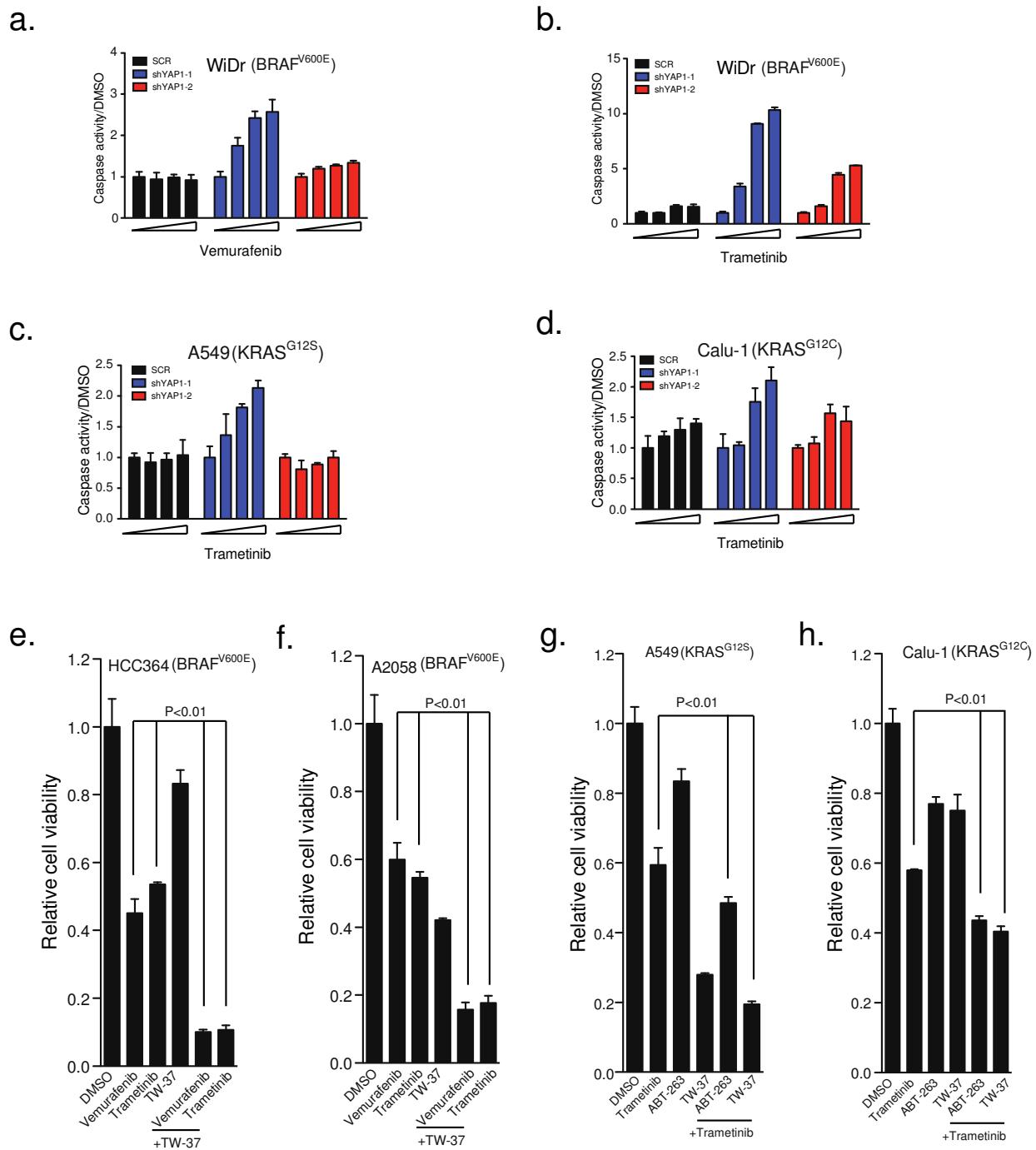


Supplementary Figure 10

YAP1 knockdown sensitizes RAS-mutant NSCLC cells to MEK inhibition.

(a–f) Effects of *YAP1* knockdown using two independent shRNAs on trametinib sensitivity in **(a)** A549 KRAS G12S–mutant, **(b)** H23 KRAS G12C–mutant, **(c)** Calu-6 KRAS G12C–mutant, **(d)** H2347 NRAS Q61R–mutant, **(e)** MOR/CPR KRAS G12C–mutant and **(f)** SW1573 KRAS G12C–mutant NSCLC cell lines. **(g–i)** Effects of *YAP1* knockdown using two independent shRNAs on trametinib sensitivity in **(g)** MOR/CPR KRAS G12C–mutant cells, **(h)** SW1573 KRAS G12C–mutant cells and **(i)** H2347 NRAS Q61R–mutant cells (shown is 7-d cell growth assessed by crystal violet staining assays, with quantification of the effects on viability under each condition).

Supplementary Figure 11.



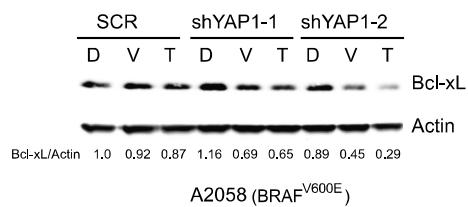
Supplementary Figure 11

***YAP1* knockdown induces apoptosis upon RAF-MEK inhibition.**

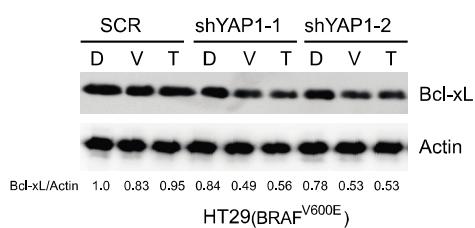
(a,b) Effects of *YAP1* knockdown on apoptosis induced upon **(a)** vemurafenib and **(b)** trametinib treatment in WiDr BRAF-mutant colon cancer cells as measured by caspase-3/7 activation. **(c,d)** Effects of *YAP1* knockdown on apoptosis induced upon **(c)** vemurafenib and **(d)** trametinib treatment in A549 KRAS-mutant lung cancer cells as measured by caspase-3/7 activation. **(e,f)** Effects of pharmacological inhibition of BCL-xL using TW-37 on vemurafenib or trametinib sensitivity in **(e)** HCC364 BRAF-mutant lung cancer cells and **(f)** A2058 BRAF-mutant melanoma cells ($n = 3, \pm$ s.e.m. for all cell viability data shown; P values are indicated for statistical analysis). **(g,h)** Effects of pharmacological inhibition of BCL-xL using ABT-263 or TW-37 on trametinib sensitivity in **(g)** A549 KRAS G12S-mutant and **(h)** Calu-1 KRAS G12C-mutant lung cancer cells ($n = 3, \pm$ s.e.m. for all cell viability data shown; P values are indicated for statistical analysis).

Supplementary Figure 12.

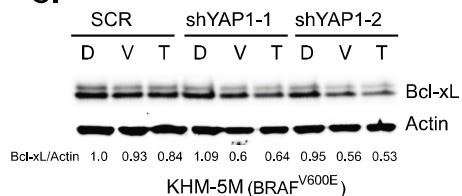
a.



b.



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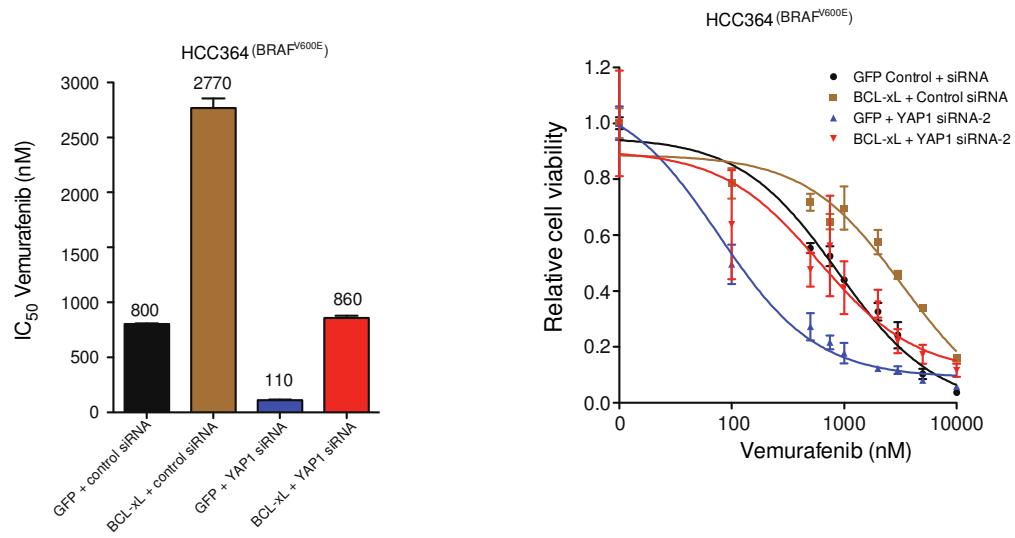
Supplementary Figure 12

YAP inhibition plus RAF-MEK inhibition suppresses BCL-xL expression.

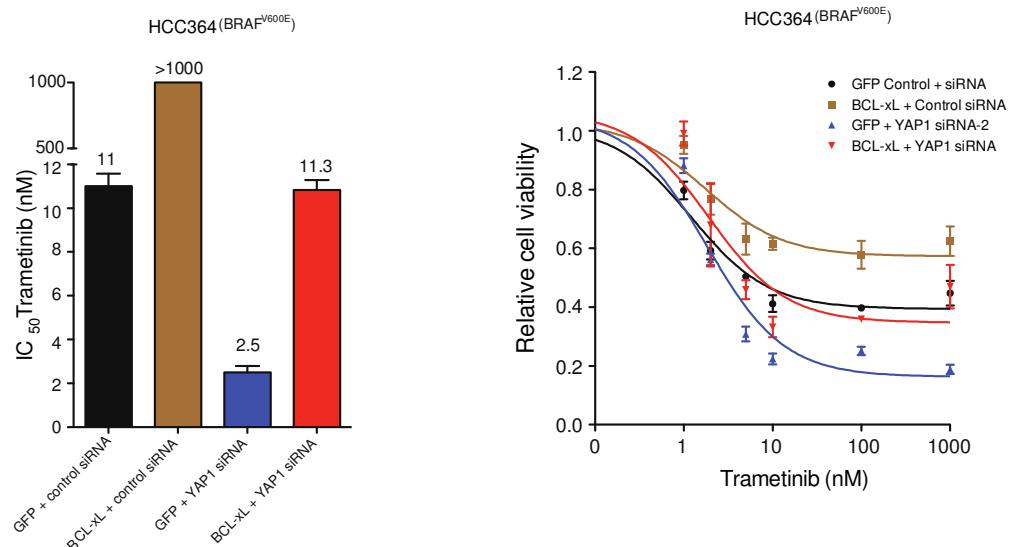
(a–c) Effects of YAP suppression and vemurafenib or trametinib treatment on BCL-xL levels by immunoblot analysis in BRAF V600E–mutant **(a)** A2058 melanoma, **(b)** HT29 colon and **(c)** KHM-5M thyroid cancer cells. Results represent three independent experiments.

Supplementary Figure 13.

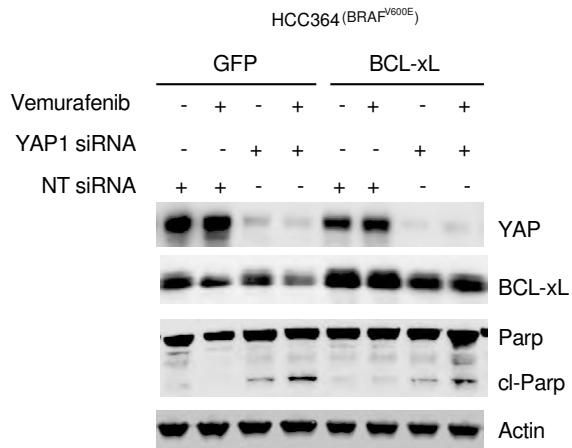
a.



b.



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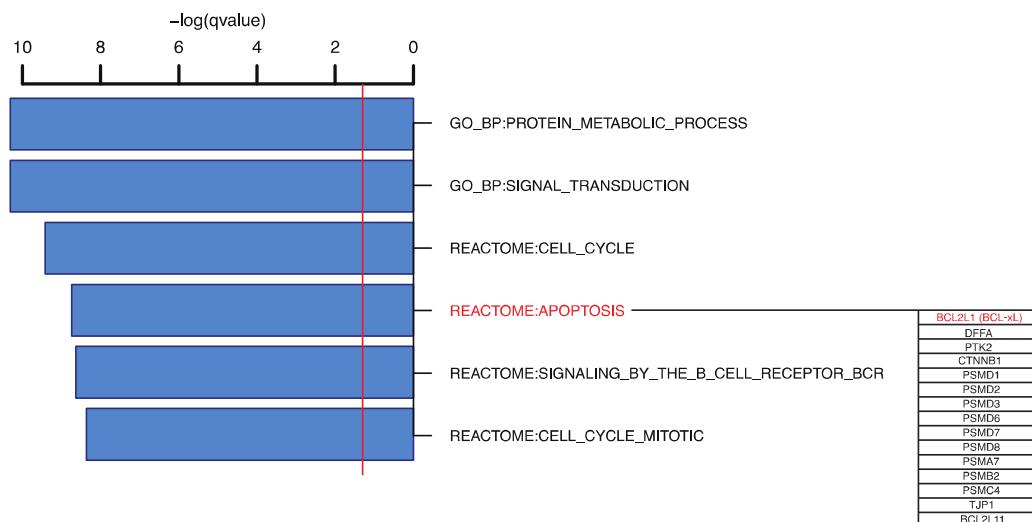


Supplementary Figure 13

BCL-xL expression rescues *YAP1*-depleted cells from growth suppression by RAF-MEK inhibition.

(**a,b**) Effects of BCL-xL overexpression and *YAP1* knockdown on (**a**) vemurafenib and (**b**) trametinib sensitivity in HCC364 lung cancer cells (shown are the IC₅₀ and relative cell viability). (**c**) Immunoblot analysis for each indicated protein in lysates from HCC364 cells overexpressing either GFP or BCL-xL and treated with either control siRNA or *YAP1* siRNA.

Supplementary Figure 14.



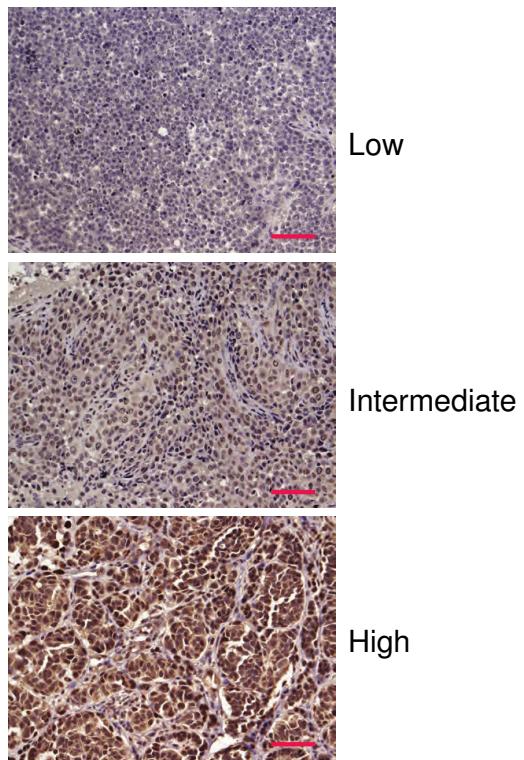
Supplementary Figure 14

Gene sets enriched among the genes specifically altered by YAP and MEK coinhibition.

Pathway analysis indicating enriched gene sets among those genes whose expression was specifically decreased by YAP and MEK coinhibition (**Supplementary Table 5**). The top six significantly represented pathways among these genes are shown (significance of pathway representation is shown as $-\log q$, with the red line indicating $q < 0.05$). The genes highlighted in the inset are the significantly altered apoptosis-related genes.

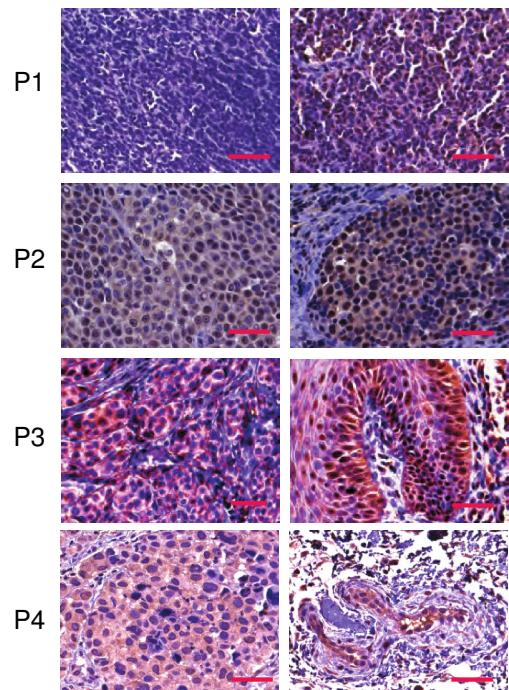
Supplementary Figure 15.

a. YAP staining



b.

Pre-treatment Acquired resistance



Supplementary Figure 15

YAP protein expression in human tumor specimens by YAP immunohistochemistry.

(a) Representative images of immunohistochemistry staining of YAP protein in representative human tumor specimens with low, intermediate and high YAP staining (brown). **(b)** YAP staining by immunohistochemistry in BRAF V600E–mutant melanomas obtained before RAF or MEK inhibitor treatment and upon acquired resistance. Shown are the 4 out of 16 cases in which the pretreatment tumor did not harbor high levels of YAP (showing either low or intermediate staining by immunohistochemistry). Bars represent 50 microns.

Supplementary Tables

Supplementary Table 1. Gene targets included in the pooled shRNA screening library.

Supplementary Table 2. Primary screening hits, using a p-value of <0.005 for depletion in vemurafenib-treated cells.

Gene	Dropout p-value
YAP1	1.00E-05
PCK1	2.00E-04
UPB1	2.00E-04
CHRNA9	3.00E-04
PLCB3	5.00E-04
SDHC	5.00E-04
WIPI1	6.00E-04
HSF2	8.00E-04
SUZ12	8.00E-04
TLN1	8.00E-04
GAS6	0.0011
SH2B2	0.0012
NDUFA10	0.0013
SLCO1A2	0.0014
SLC13A5	0.0015
HCLS1	0.0018
BIRC5	0.002
MAP3K3	0.0021
PARP1	0.0025
MVP	0.0028
TNFAIP3	0.0028
NCOR1	0.0029
SFRP2	0.003
PKMYT1	0.0031
LDB1	0.0033
TOP3B	0.004
GAB1	0.0042
ST6GALNAC6	0.0046

Supplementary Table 3. IC₅₀ of cell lines to BRAF inhibitor vemurafenib and MEK inhibitor trametinib infected with scramble shRNA or YAP1 shRNA.

Cell lines	Genotypes	Vemurafenib IC ₅₀ SCR/shYAP1-1/shYAP1-2	Trametinib IC ₅₀ SCR/shYAP1-1/shYAP1-2
HCC364	BRAF ^{V600E}	800/ 100/200	10/ 2.1/4.03
A2058	BRAF ^{V600E}	>5000/ 1500/2000	500/ 1.5/2
WM793	BRAF ^{V600E}	3000/ 800/600	>1000/ 3/3
HT29	BRAF ^{V600E}	>10000/ 6000/6000	75/ 10/10
WiDr	BRAF ^{V600E}	2500/ 200/700	2/ 0.5/0.5
KHM-5M	BRAF ^{V600E}	>10000/ 3000/5000	>1000/ 2.5/25
HTC/C3	BRAF ^{V600E}	>5000/ 200/2500	200/ 2/7
Cal-12T	BRAF ^{G466V}		723/ 10.1/15.3
A549	KRAS ^{G12S}		75/ 30/30
H23	KRAS ^{G12C}		75/ 15/25
Calu-6	KRAS ^{G12C}		50/ 15/5
H2347	NRAS ^{Q61R}		9/ 2/3
SW1573	KRAS ^{G12C}		>1000/ 200/180
MOR/CPR	KRAS ^{G12C}		>1000/ 150/150
MM415	NRAS ^{Q61L}		1.767/ 0.77/0.85
SK-MEL-2	NRAS ^{Q61R}		1.55/ 0.75/1
HPAF-II	KRAS ^{G12D}		9/ 3.6/4.3
PANC 02.03	KRAS ^{G12D}		40/ 6/15

Supplementary Table 4. Percentage of maximal growth inhibition upon MEK inhibitor trametinib treatment in cell lines infected with scramble shRNA or YAP1 shRNA.

Cell lines	Genotypes	Trametinib maximal growth inhibition SCR/shYAP1.1/shYAP1-2
HCC364	BRAF ^{V600E}	64.3%/94.6%/94.7%
A2058	BRAF ^{V600E}	62%/84%/80%
WM793	BRAF ^{V600E}	50%/76%/73%
HT29	BRAF ^{V600E}	81%/88%/90%
WiDr	BRAF ^{V600E}	91%/96%/96%
KHM-5M	BRAF ^{V600E}	48%/85%/74%
HTC/C3	BRAF ^{V600E}	54%/67%/65%
Cal-12T	BRAF ^{G466V}	53%/71%/72%
A549	KRAS ^{G12S}	60%/82%/77%
H23	KRAS ^{G12C}	68%/86%/82%
Calu-6	KRAS ^{G12C}	70%/94%/85%
H2347	NRAS ^{Q61R}	70%/94%/82%
SW1573	KRAS ^{G12C}	42%/61%/65%
MOR/CPR	KRAS ^{G12C}	35%/73%/81%
MM415	NRAS ^{Q61L}	89%/96%/95%
SK-MEL-2	NRAS ^{Q61R}	92%/99%/95%
HPAF-II	KRAS ^{G12D}	89%/95%/96%
PANC 02.03	KRAS ^{G12D}	84%/93%/92%

Supplementary Table 5. Differentially expressed genes in BRAF^{V600E} NSCLC cells (HCC364), upon either YAP knockdown alone (by shRNA), trametinib treatment alone, and combined YAP knockdown + trametinib treatment. Gene name, fold change in expression (compared to scramble control), and adjusted p-value are listed for each gene that was significantly altered under any of the three conditions using the following significance criteria: adjusted p-value < 0.05 and at least 40% decreased expression or at least 66% increased expression, compared to the control. Columns H, I, J indicate whether each gene significantly altered under any condition was significantly altered in the other conditions. N = not altered. Y = altered. The genes specifically significantly altered by combined YAP knockdown + trametinib treatment (those with ‘N’ for both YAP knockdown and trametinib treatment alone and ‘Y’ for combined YAP knockdown and trametinib) were subjected to pathway analysis (shown in Figure S15).

Supplementary Table 6. YAP1 expression as measured and scored by IHC in the human clinical specimens with complete response (CR) or incomplete response (IR, including partial response/PR and stable disease/SD) to MAPK pathway inhibitor treatment.

Patients	Treatment	Type of responses	YAP score	Cancer types
1	vemurafenib	CR	low	melanoma
2	vemurafenib	CR	low	melanoma
3	vemurafenib	CR	intermediate	melanoma
4	vemurafenib	CR	high	melanoma
5	dabrafenib + trametinib	CR	intermediate	melanoma
6	vemurafenib	CR	intermediate	melanoma
7	vemurafenib	IR (PR)	low	melanoma
8	dabrafenib	IR (PR)	low	melanoma
9	dabrafenib	IR (PR)	high	melanoma
10	vemurafenib	IR (PR)	high	melanoma
11	dabrafenib	IR (PR)	high	melanoma
12	dabrafenib + trametinib	IR (PR)	high	melanoma
13	dabrafenib	IR (PR)	high	melanoma
14	dabrafenib + trametinib	IR (PR)	intermediate	melanoma
15	dabrafenib + trametinib	IR (PR)	high	melanoma
16	dabrafenib + trametinib	IR (PR)	high	melanoma
17	dabrafenib + trametinib	IR (PR)	high	melanoma
18	dabrafenib + trametinib	IR (PR)	high	melanoma
19	dabrafenib + trametinib	IR (PR)	high	melanoma
20	dabrafenib + trametinib	IR (PR)	high	melanoma
21	dabrafenib + trametinib	IR (PR)	high	melanoma
22	dabrafenib + trametinib	IR (PR)	high	melanoma
23	dabrafenib + trametinib	IR (SD)	high	melanoma
24	dabrafenib + trametinib	IR (SD)	high	melanoma
25	dabrafenib + trametinib	IR (SD)	high	melanoma
26	vemurafenib	IR (PR)	intermediate	melanoma
27	vemurafenib	IR (PR)	high	melanoma
28	vemurafenib	IR (PR)	high	melanoma
29	vemurafenib	IR (SD)	high	melanoma
30	vemurafenib	IR (SD)	high	melanoma
31	LGX818+MEK162	IR (SD)	intermediate	melanoma
32	LGX818+MEK162	IR (SD)	high	melanoma
33	LGX818+MEK162	IR (PR)	high	melanoma
34	LGX818+MEK162	IR (PR)	high	melanoma
35	LGX818+MEK162	IR (PR)	high	melanoma
36	dabrafenib	IR (PR)	intermediate	NSCLC
37	dabrafenib	IR (PR)	high	NSCLC
38	dabrafenib	IR (PR)	high	NSCLC
39	dabrafenib	IR (PR)	high	NSCLC
40	dabrafenib	IR (PR)	high	NSCLC

Supplementary Table 7. Melanoma patient information for acquired RAF-MEK inhibitor resistance analysis (average TTP of 9.9 months, consistent with prior studies¹⁻⁴).

Patients	Treatment	YAP score		Time to progression (TTP)
		Pretreatment	Acquired resistance	
P1	vemurafenib	Low	High	17 months
P2	vemurafenib	Intermediate	High	10 months
P3	vemurafenib	Intermediate	High	21 months
P4	dabrafenib + trametinib	Intermediate	High	17 months
P5	dabrafenib + trametinib	High	High	9 months
P6	vemurafenib	High	High	2 months
P7	dabrafenib + trametinib	High	High	3 months
P8	dabrafenib + trametinib	High	High	2 months
P9	vemurafenib	High	High	5 months
P10	dabrafenib + trametinib	High	High	30 months
P11	dabrafenib	High	High	7 months
P12	dabrafenib + trametinib	High	High	10 months
P13	vemurafenib	High	High	8 months
P14	dabrafenib + trametinib	High	Intermediate	N/A
P15	dabrafenib + trametinib	High	Intermediate	3 months

P16	vemurafenib	High	Low	5 months
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Supplementary references

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